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World J Gastroenterol 2007 August 14; 13(30): 4035-4041
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

Enteric glial cells and their role in gastrointestinal motor abnormalities: Introducing the neuro-gliopathies

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Received: 2007-04-07 Accepted: 2007-05-12

Abstract

The role of enteric glial cells has somewhat changed from that of mere mechanical support elements, gluing together the various components of the enteric nervous system, to that of active participants in the complex interrelationships of the gut motor and inflammatory events. Due to their multiple functions, spanning from supporting elements in the myenteric plexuses to neurotransmitters, to neuronal homeostasis, to antigen presenting cells, this cell population has probably more intriguing abilities than previously thought. Recently, some evidence has been accumulating that shows how these cells may be involved in the pathophysiological aspects of some diseases. This review will deal with the properties of the enteric glial cells more strictly related to gastrointestinal motor function and the human pathological conditions in which these cells may play a role, suggesting the possibility of enteric neuro-gliopathies.

Key words: Enteric glia; Glial cells; Gastrointestinal motility

Bassotti G, Villanacci V, Fisogni S, Rossi E, Baronio P, Clerici C, Maurer CA, Cathomas G, Antonelli E. Enteric glial cells and their role in gastrointestinal motor abnormalities: Introducing the neuro-gliopathies. *World J Gastroenterol* 2007; 13(30): 4035-4041

http://www.wjgnet.com/1007-9327/13/4035.asp

INTRODUCTION

The enteric nervous system (ENS) is organized in a complex structure that controls motility, blood flow, uptake of nutrients, secretion, immunological and inflammatory processes in the gut[1]. Two main cell populations are represented in the ENS, neurons and enteric glial cells (EGC), the latter being much more abundant (up to fourfold) than neurons[2,3] (Figure 1). In humans, the ENS is subdivided into several plexuses (subserous, longitudinal muscle, myenteric, circular muscle, deep muscular, muscularis mucosae, and mucosal)[4]. Ganglionated plexuses are present in the submucosa (Meissner's and Henle's plexuses) and in the septum between the circular and longitudinal layers of the muscularis propria (Auerbach's plexus)[5] (Figure 2A). Most EGC are found within the ganglia, and are also present in the interconnecting nerve strands of the ganglionated and in all non-ganglionated plexuses[6,7].

In the time course, the traditional view of EGC function has changed from simple mechanical support (as their very name, derived from the Greek "glue", implies) to more articulate and complex ones, extremely important for the homeostasis of the gut, including influence on motility and inflammatory processes[8-10].

In this article we will take into consideration the role of EGC, looking at both experimental animal models and some human diseases for which evidence exists, and in particular their involvement in intestinal motor abnormalities and inflammatory conditions of the gut.

MORPHOLOGY AND IDENTIFICATION OF EGC

Anatomical considerations

These cells are small, with several projecting processes of various length and shapes, which often confer them a star-like appearance[2,11,12] (Figure 2B). In the ganglia, EGC are very tightly packed around neurons[2,13] (Figure 3) and extend several flat projections which incompletely insulate enteric neurons from extraganglionic cells[2,14,15], whereas in the nerve strands glial processes wrap up several axonal bundles[2,16]. Electron microscopic studies have shown that EGC contain intracellular arrays of 10 nm filaments (mainly constituted by glial fibrillary acidic protein, GFAP[17-19] crisscrossing their bodies, forming axial bundles and anchoring the cells to the ganglionic surfaces[2] (Figure 4).

Moreover, some studies have suggested that EGC in the various plexuses layers of the ENS may be constituted by

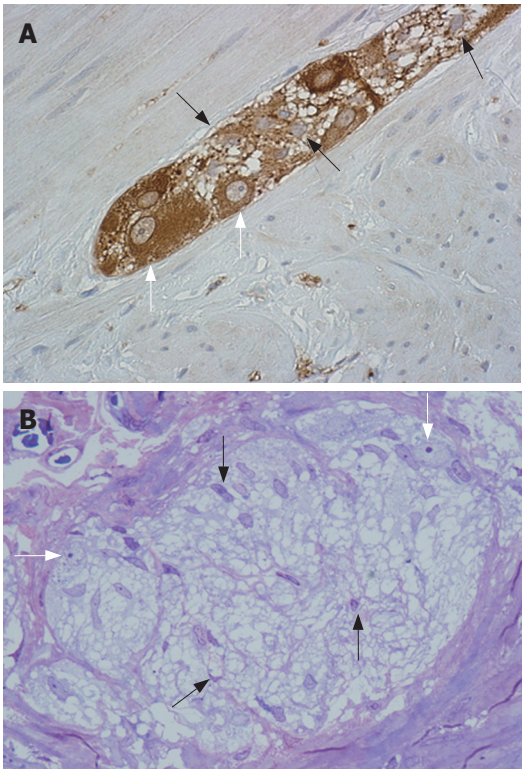

Figure 1 **A**: Human colonic myenteric plexus showing that neurons (white arrows) are less numerous with respect to EGC (black arrows) (NSE immunostaining, x 40); **B**: Semithin section of human colonic submucosal plexus, showing the preponderance of EGC (black arrows) with respect to the enteric neurons (white arrows) (Toluidine blue, x 40).

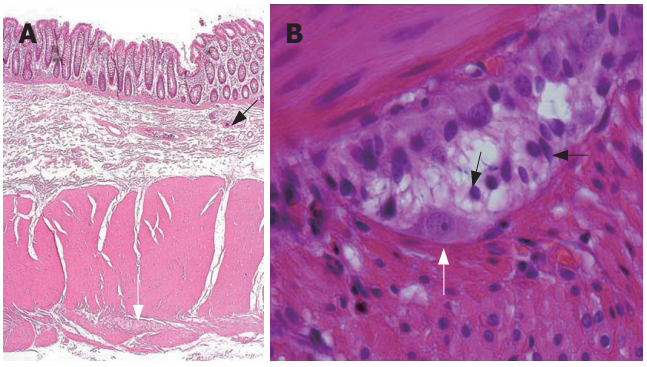

Figure 2 **A**: Full thickness section of the human colon, showing the submucosal (black arrow) and the myenteric plexus (white arrow) (HE, x10); **B**: Human myenteric ganglion, showing numerous EGC (black arrows) and an enteric neuron (white arrow) (HE, x 100).

functionally heterogeneous populations[9,20-22].

Histological and immunohistochemical considerations

The EGC were first described in 1899 with methylene blue staining on full thickness preparations[23]; today, immunohistochemical methods are most frequently employed for their identification. Mature EGC strongly express vimentin[24] (also expressed in myofibroblasts)[25] and GFAP, considered a specific gut glial marker[17] even though its cellular functions are still obscure[26]. Another frequently used EGC marker is the S100 protein, which is thought to yield the best results in identifying these cells[27].

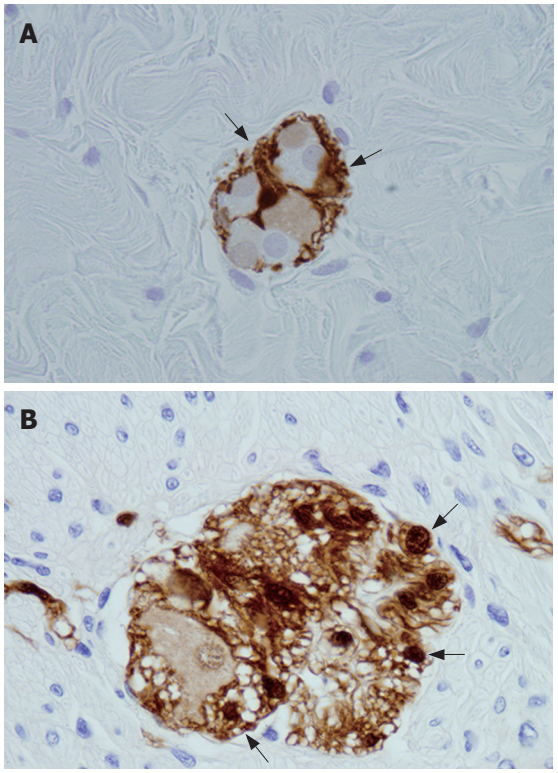

Figure 3 EGC (arrows) tightly packed around enteric neurons in a submucosal (**A**) and a myenteric ganglion (**B**) (S100 immunostaining, x 40).

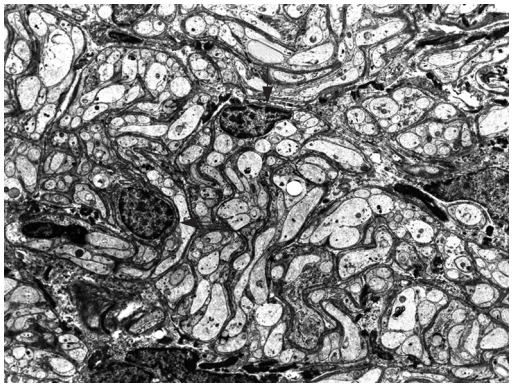

Figure 4 Electron microscopic image of glial cells (arrows) in a human colonic myenteric ganglion (x 1900).

This protein regulates cytoskeletal structure and function and calcium homeostasis in the cytoplasm of glial cells[28]; in the ENS S100 is thought to be exclusively localized in these cells[29]. Other putative antibodies for EGC, such as glutamine synthetase (GS)[30] and the glial cell surface antigen Ran-2[31] have not been widely employed.

It is also important to underline the fact that EGC, being of neuroectodermal origin[32], are not related to microglia in the central nervous system, which has a monocyte-macrophage lineage[33]. Instead, EGC share more similarities with astrocytes, the predominant glial cells of the central nervous system, that regulate synaptic transmission and neurovascular coupling[34], in addition to be of paramount importance for the formation and function of the blood-neural barrier[35]. However, it must

be stressed that although EGC display some similarities with astrocytes, there are differences between these two cell populations, such as the dependence on neuregulins in EGC[9,36] and the functional properties[9,25]. Finally, the EGC are structurally and functionally different from the Schwann cells of the peripheral nerves (including those within the intestinal wall)[37,38]; Schwann cells of intramural nerves are S-100-positive but GFAP-negative[39].

FUNCTIONS OF EGC

Homeostatic function

Experimental evidence suggests that EGC are essential for maintaining the homeostasis of enteric neurons. Studies in animal models demonstrated that the loss of enteric glia causes neuronal degeneration[40,41] and/or alterations of the neurochemical coding of enteric neurons[42]. It is thought that the structural and functional integrity of enteric neurons may be due to the glial synthesis of some still unknown trophic factor[43]. Some studies, for instance, have shown that mature EGC produce glial-derived neurotrophic factor and neurotrophin-3[44-46], even though no neuronal populations depending on these substances have so far been described in the ENS of mammals. Recent investigations have shown that EGC may have a dipeptide transport function, contributing to the clearance of neuropeptides in the ENS[47].

ENS mechanical supporting functions

EGC are anchored to the surface of enteric ganglia and nerve strands by means of GFAP bundles[48], and respond to mechanical stimulation increasing the expression of the immediate-early c-fos gene[49], raising intracellular Ca^{2+} levels and spreading intercellular Ca^{2+} waves[50]. Thus, it is thought that these cells support and stabilize the ENS through continuous adaptations to the structural and metabolic impairments of the gut wall[9]. Moreover, EGC express voltage-activated inward and outward K^+-channels[51], suggesting a possible role in preventing extracellular accumulation of K^+, which can impair synaptic transmission and ion channel kinetics in the ENS.

Neurotransmitter function

EGC might also be involved in the enteric neurotransmission. In fact, due to the exclusive expression of GS by these cells[24,30], and the presence of glutamate immunoreactivity in human EGC[52], enteric glia could have a role in glutamatergic signaling[9] and represents a source of glutamine for neuronal glutamate and gamma-aminobutyric acid (GABA) resynthesis[53]. This is further supported by the demonstration that immunoreactivity to the high-affinity GABA transporter GAT2 mostly occurs in EGC[54], suggesting that the latter might rapidly remove GABA from the extracellular space. Moreover, since EGC but not enteric neurons display immunoreactivity to L-arginine (an essential precursor for nitric oxide)[55,56], a role in nitrergic neurotransmission might also be possible.

Owing to the fact that EGC propagate intercellular Ca^{2+} waves, an orchestrated intestinal glial activity has been postulated[50] that would act through a functional network[57,58]. This network is suggested by the demonstration of cell-to-cell coupling between EGC (probably through gap junctions)[12,50,51,58] and the expression of the P2Y4 receptor on these cells[59]. EGC may also transfer information to the neurons by means of nucleotide signaling[60-62]. Numerous molecules (serotonin[60], histamine[60], endothelin[63], protease-activated receptors[64]) can also activate EGC, which increase intracellular Ca^{2+} concentrations[65] or express the *c-fos* gene, a marker of early cell activation. It has also been shown that EGC express purinoreceptors[60,66] and that multiple lipid-activated signalling mechanisms exist in these cells[67,68].

EGC AND GASTROINTESTINAL INFLAMMATION/MOTILITY

EGC and intestinal inflammation

Experimental animal studies have demonstrated that EGC may have a role in intestinal inflammatory processes[9], and that initiation and/or progression of inflammatory bowel disease (especially Crohn's disease) might be ascribed to an immune-mediated damage to enteric glia[69]. The fact that EGC functionally interact with lymphocytes[70-73], respond actively to inflammation, and become activated as antigen-presenting cells[74] attracting immune cells to the ENS[9,75], suggests that this cell population is likely involved in inflammatory processes of the gut. Moreover, the immune cells are usually nearby, since the intestine physiologically contains such a cell population that provides a series of pattern recognition receptors interacting with bacterial molecular patterns, and helps to modulate intestinal innate immunity and an appropriate adaptive immune response[76-78].

Thus, it is not difficult to imagine these cells as active participants in the pathogenesis of the so-called "functional" gastrointestinal disorders. These are usually thought to occur in the absence of anatomical or biochemical abnormalities[79]. However, this definition now seems outdated, because structural and molecular abnormalities have begun to be recognized in subsets of patients[80]. For instance, studies in patients with irritable bowel syndrome disclosed the presence of inflammatory infiltrates closely associated with the enteric plexuses and mucosal activation of the immune system[81,82], and some patients with intestinal dysmotility and megacolon have a lymphoplasmacellular infiltrate within the myenteric plexus that likely accounts for their symptoms[83].

Evidence for involvement of EGC in abnormal gastrointestinal motility

The role of EGC has been investigated in only a few human diseases, even though there is still no pathological condition entirely ascribable to EGC dysfunction. For instance, patients with colonic diverticular disease have a significant decrease of EGC and of interstitial cells of Cajal (ICC) in the enteric plexuses[84]. Owing to the fact that in colonic diverticulosis the smooth muscle hypertrophy acts as a partially obstructive mechanism, the EGC population loss might be partly due to this mechanism, similar to that documented for ICC in analogous experimental animal models[85].

The number of EGC, together with that of enteric neurons and ICC, is also considerably decreased in patients

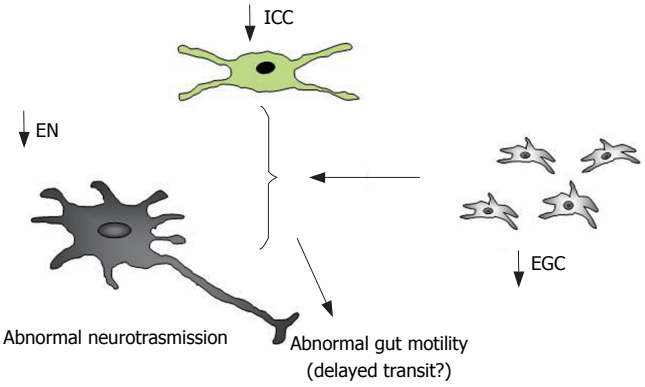

Figure 5 Putative mechanisms linked to the decrease of enteric glial cells (EGC), leading to abnormal gut motility. EN: enteric neurons; ICC: interstitial cells of Cajal.

with severe constipation (slow-transit type) undergoing surgery for intractable symptoms[86]. Interestingly, the loss of EGC, but not of ICC and enteric neurons, was also documented in the terminal ileum of these patients[87]; this implies that this cell population may be involved in the small bowel dysmotility repeatedly described in these patients[88,89]. A significant decrease of EGC, but not of other elements of the ENS, was then described in the myenteric and submucosal plexuses of patients with severe constipation due to obstructed defecation refractory to medical treatment and biofeedback training[90]. These findings are intriguing, and consistent with the recent hypothesis, based on abnormal colonic manometric findings, that at least one subpopulation of patients with obstructed defecation might result from defective colonic, rather than anorectal, function[91]. Of practical importance, our results could give an explanation for the lack of response to treatments, especially to biofeedback, in these patients.

More recently, we have reported a significant decrease of EGC in patients with chagasic and idiopathic megacolon compared to controls[92]; it is worth noting that all the above human pathological conditions in which EGC have been found to be decreased share a common denominator, i.e. constipation.

How can we explain the role of EGC in pathological conditions?

We could hypothesize that the reduced number of EGC, together with the decrease/loss of other cell populations essential for gastrointestinal motility, may play a role in these diseases. For instance, the ICC decrease impairs the pacemaker enteric signals and might add to an abnormal neurotransmission secondary to the decreased number of enteric neurons, and be further worsened by an impairment of EGC. The summation of the loss of the properties of these cell populations might thus lead to dysmotilities of the involved viscera, by means of several mechanisms (Figure 5): impairment of the mechanical properties of the plexuses, decreased gut neurotransmission, and reduced homeostatic support to the enteric neurons, leading to neurodegeneration and/or phenotypic shift, even in the absence of inflammation[9,93]. Experimental animal models also support this hypothesis, suggesting that EGC play a major role in the modulation of enteric neural circuits that regulate intestinal motility[94].

Why EGC, ICC, and enteric neurons are decreased in such patients is still unknown. Evidences in experimental animal models suggest that the number of EGC reduces with aging[95], but this has not been evaluated in human beings[96]. Other mechanisms, such as the damaging effect of antraquinone laxatives on the ENS, have not been confirmed with modern immunohistochemical methods[97].

CONCLUSION

Probably, the EGC should be looked at differently, since evidence is mounting concerning an ever more active role in the complex organization of the gastrointestinal tract, including enteric neuroplasticity[98]. The (limited) data so far accumulated suggest that these cells are probably somewhat involved in some motor dysfunction of the gastrointestinal tract, mainly those characterized by constipation. Thus, it is likely that in the future other "functional" disorders of the gut, in addition to the irritable bowel syndrome, may be reclassified. For instance, we have recently proposed consideration of at least some subtypes of constipation such as enteric neuropathies[99], although seen in the light of the data on EGC we should probably reformulate this definition in terms of neurogliopathies. However, more evidence are needed to establish a more precise role for this fascinating cell population, especially considering new perspectives, such as the possibilities of neural stem cell transplantation[100-102] for the treatment of disorders of the peripheral and central nervous system. Hopefully, studies on EGC will possibly be useful to establish new therapeutic approaches to some gut disorders.

ACKNOWLEDGMENTS

We are warmly grateful to Professor Giorgio Gabella, Department of Anatomy and Developmental Biology, University College London, London, UK, for kindly providing useful bibliographic references.

COMMENTS

Background

Enteric glial cells (EGC) are to date thought to be more than simple support structures for the enteric nervous system (ENS). Recent developments in their biological properties led to the belief that this cell population may have pathophysiological importance in inflammatory and dysmotility conditions of the gut.

Research frontiers

The EGC have also, in addition to mechanical support function in the ENS, homeostatic functions (are essential for enteric neuronal vitality), neurotransmitter functions, immunological functions (may act as antigen-presenting cells) and appear critically involved in the pathophysiology of inflammatory bowel diseases, especially Crohn's disease.

Innovations and breakthroughs

Recent research in human beings showed that the EGC are likely involved also in the pathophysiological mechanisms of some diseases presenting with abnormal gastrointestinal motility, and especially those characterized by constipation. In fact, significant decreases of EGC have been reported in diverticular diseases, slow transit constipation, some subsets of obstructed defecation, Chagasic and idiopathic megacolon.

Applications

The study of EGC, in addition to that of other components of the ENS (neurons, interstitial cells of Cajal), might result in a better understanding of the pathophysiological grounds of conditions characterized by abnormal gut activity, and perhaps lead to a more targeted therapeutic approach to these disorders.

Peer review

This is an interesting review dealing with the properties of the enteric glial cells, more strictly related to gastrointestinal motor function and the human pathological conditions in which these cells may play a role, suggesting the possibility of enteric neuro-gliopathies.

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